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Review article

Roles of integrin-linked kinase in cell signaling and its perspectives as a therapeutic target

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A B S T R A C T

Integrin-linked kinase (ILK) localizes to focal adhesions, and interacts with the cytoplasmic tail of β subunits of integrins and couples them to the actin cytoskeleton. ILK may act as a kinase and transmit the signals in a phosphatidylinositol 3-kinase-dependent manner, or can act as a scaffold protein to function through cell–matrix interactions, cell signaling, and cytoskeletal organization. Within this pivotal position, ILK mediates many important cellular processes, including survival, proliferation, differentiation, adhesion, migration, contractility, etc. Besides, ILK plays some role in the activation of endothelial progenitor cells and neovascularization, and may also enhance vascular endothelial growth factor expression. Increased ILK activity may promote epithelial-to-mesenchymal transition and induce a transformed, tumorigenic phenotype. Higher expression of ILK was frequently noted in human malignancies. ILK may also be important for mitotic-spindle assembly. Inhibition of ILK causes proliferative defects, induces cell-cycle arrest and apoptosis, and is embryonically lethal. New concepts of gene or cell-based therapy working on the up- or downregulation of ILK have emerged as a valid therapeutic approach for cancer treatment, and also a new hope for vasculogenesis in the ischemic area. The current review will discuss some known mechanisms, and the role of ILK in the modulation of tumorigenesis and reproduction, based on an extensive literature survey.

Introduction

Integrin-linked kinase (ILK) is a key scaffold protein that localizes to focal adhesions, acts as a central component of a heterotrimer (the ILK–PINCH–parvin complex). Since its discovery, ILK has been demonstrated to have an essential role in connecting the cytoplasmic tail of β subunits of integrins to the actin cytoskeleton, and in regulating actin polymerization.[1] Within this pivotal position, ILK has been shown to interact with many intracellular proteins through PINCH or parvin to mediate diverse arrays of biological events,[2] or to mediate cell responses induced by the interaction of integrins with the extracellular matrix (ECM).[2,3] ILK is involved in the regulation of cell growth, survival, adhesion, invasion, and migration. Increased ILK activity may promote epithelial–mesenchymal transition (EMT)[4] and angiogenesis; aberrantly overexpressed or activated ILK has also been found in many types of human malignancies.[5]

The female reproductive system is strongly governed and influenced by the cyclic ovarian hormones, and the endometrium exhibits rapid cyclical shedding and regrowth throughout the female reproductive life. During the menstrual cycle, the endometrium undergoes cell proliferation and then decidualization, a process of tissue remodeling for the preparation of embryo implantation that includes molecular differentiation, morphological transformation, ECM reorganization, as well as variations in integrin moiety expression, which is called "integrin switching."[6] Three integrins, α1β1, α4β1, and αvβ3, coexpress at the window of implantation.[7,8] In addition, endometriosis and adenomyosis, which are common pathologies of the female reproductive system that induces significant pelvic pain and subfertility, are caused by

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the ectopic spread and growth of the endometrium; however, the exact mechanism of pathogenesis remains elusive despite many research efforts.[9] Although endometriosis and adenomyosis are generally thought to be benign diseases, malignancy can occur unexpectedly in rare situations.[10]

As ILK acts in the center stage of cell–matrix adhesion, and a cyclic integrin switching takes place in endometrial cells throughout the menstrual cycle, it is reasonable to assume that ILK can play some important roles in endometrial functions of decidualization and implantation, as well as in its unique pathology of ectopic spread, or even in malignant transformation. In the current review, a literature search was conducted to discuss some known mechanisms by which ILK functions, the possible roles of ILK in tumorigenesis and reproduction, and some prospects of ILK as a therapeutic target.

ILK is in the center stage of cell–matrix adhesion and signaling

Integrins are the major cell surface receptors that recognize and bind ECM proteins, on which the communication between the cells and ECM is based. Integrins are the prototypic transmembrane receptors that induce the formation of focal adhesions. Upon binding to its extracellular ligand, integrin would induce intracellular signaling processes, involving molecules such as ILK or focal adhesion kinase.[11]

ILK is a highly evolutionarily conserved intracellular protein,[12] and is required for embryogenesis and tissue homeostasis, as mice with ILK-conditional knockout are embryonic lethal shortly after implantation due to defective epiblast polarization and abnormal F-actin accumulation.[13]

Although there is a consensus that ILK is biologically important, it remains controversial as to how ILK can confer its functions. Recent structural, biochemical, and genetic analyses have revealed that the protein kinase domain of ILK lacks some amino-acid residues thought to be essential for phosphotransferase activity; instead, it contains multiple pseudoactive site features, including the unusually short and rigid activation segment that lacks a conserved phosphorylation site, altered magnesium coordination topology, and a severely degraded catalytic loop. Although initially named as a kinase, ILK is frequently questioned to be a pseudokinase.[2]

ILK may function as an adaptor/scaffold protein

Evidence has shown that ILK, a central piece of the ILK–PINCH–parvin complex in connecting integrins to the actin cytoskeleton and other signaling proteins, functions primarily as an adaptor/scaffold protein and works through protein–protein interactions.[12] Dynamic interactions between cells and their environments can also regulate their morphogenesis and functions. Through the protein–protein interaction mechanism, the ILK scaffold transduces signals through its attachment to focal adhesions and reorganization of the actin cytoskeleton and catalytic proteins, and thereby regulates focal adhesion assembly, cytoskeleton organization, and signaling (Fig. 1). ILK is activated through cellular interactions with the ECM and growth factors to mediate many intracellular functional effects and cellular processes, including growth, proliferation, survival, differentiation, migration, invasion, and angiogenesis.[14,15] Several studies have reported proliferative defects upon ILK depletion.[16] ILK also has central roles to play in cardiac and smooth-muscle contractility, and ILK dysregulation causes cardiomyopathies in humans.[12] Increased ILK activity may induce a transformed, tumorigenic phenotype, and may also enhance vascular endothelial growth factor (VEGF) expression, whereas inhibition of ILK induces apoptosis and cell-cycle arrest.[5]

ILK interacts directly with cytoplasmic domains of the β1 or β3 integrin subunits.[15] Integrins (α1β1, α4β1, and αvβ3) coexpress in the endometrium at the window of implantation,[7,8] and the expression of integrin α3β1 is also increased in cancer progression[17]; these integrins may be correlated to the ILK expression. ILK regulates transmembrane signals bidirectionally,[18,19] as they also modulate extracellular fibronectin fibrillogenesis[20] and fibronectin matrix assembly and deposition.[21,22]

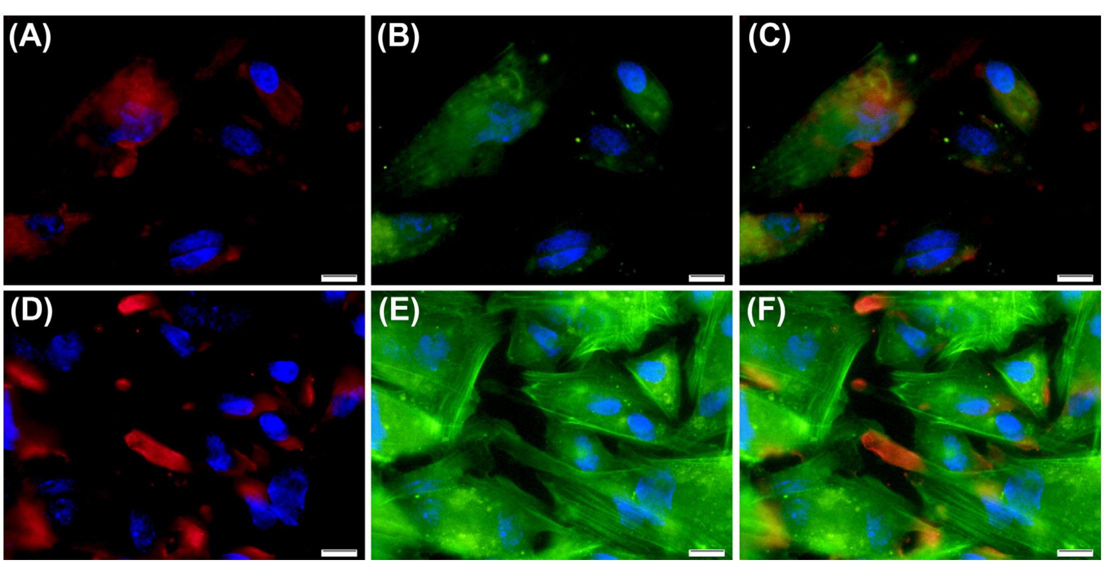

Fig. 1. Immunofluorescent staining of the intracellular distribution of ILK and actin fibers. Representative micrographs are ESCs obtained in one isolation and cultured (A–C) without *in vitro* decidualization (treated with estradiol) and (D–F) with *in vitro* decidualization (treated with estradiol plus medroxyprogesterone acetate) for 8 days. ILK was stained with Texas-red labeling (in red), intracellular actin stress fibers with phalloidin-FITC (in green), and the nucleus with DAPI (in blue). ILK in ESC without decidualization was expressed in a disperse fashion and the staining was fainter; however, in ESC with decidualization, the expression of ILK was significantly increased and more assembled (shown in a dot or spot fashion). Bar = 10 μm. DAPI = 4',6-diamidino-2-phenylindole; ESC = endometrial stromal cell; ILK = integrin-linked kinase.

ILK may function as a kinase

As the debate went on, many recent *in vitro* studies showed that ILK regulates phosphorylation of several key signaling intermediates, including phosphorylating protein kinase B (AKT1) and glycogen synthase kinase-3b (GSK3B), and the phosphotransferase activity of highly purified, full-length ILK. Therefore, ILK is now accepted as a protein kinase, at least *in vitro*.[12]

It has previously been demonstrated that ILK achieves its functions by controlling the phosphorylation of various downstream proteins, such as protein kinase B/Akt. ILK activates a range of signaling pathways downstream of integrins through its serine/threonine kinase activity. ILK transmits the signals in a phosphatidylinositol 3-kinase (PI3K)-dependent manner,[23,24] during which it regulates processes such as cell proliferation, survival, migration, and invasion.[25] Specifically, ILK phosphorylates and activates Akt at Ser 473 that regulates the genes essential for survival.[15,26] ILK also directly phosphorylates GSK3β at Ser 9, inactivating it and leading to activation of some transcription factors such as AP-1, β-catenin/Tcf, and CREB. Induction of AP-1 also stimulates the expression of matrix metalloproteinase (MMP)-9, which implicates ILK in the process of invasion and metastasis; induction of β-catenin/Tcf and CREB stimulates the overexpression of cyclin D1, which in turn induces cell proliferation.[15,27] Therefore, ILK acts as a multifunctional serine/threonine kinase and regulates the functions of cell-cycle progression, survival, migration, and invasion.

ILK functions contradictorily as both a proto-oncogene and a tumor suppressor

Conventionally, ILK was noted with increased expression and activity in many types of human malignancies and seemed to increase with tumor grades, such as the gastric cancer, breast cancer, and bladder cancer.[28] In some of these cancer types, the expression level could predict the poor outcome of the patient,[12] and inhibition of ILK has been shown to inhibit the growth, migration, invasion, and angiogenesis of cancer cells.[29] Hence, in some situations, ILK can be considered as a proto-oncogene.

However, some recent studies revealed opposing functions of ILK in the regulation of cell proliferation, differentiation, and apoptosis. One example is that ILK acts as an oncogene in the highly aggressive pediatric alveolar rhabdomyosarcoma, but contradictorily as a tumor suppressor in the embryonal rhabdomyosarcoma. The mechanism by which this occurred was found to involve the kinase activity of ILK to interact with a JNK/c-Jun pathway, which was altered by *PAX3-FKHR*, the aggressive pediatric alveolar rhabdomyosarcoma-specific chromosomal fusion gene. The expression of PAX3-FKHR would downregulate JNK1, suppress the downstream signaling of JNK1/c-Jun, and cause ILK to behave as an oncogene; however, restoration of JNK1 in aggressive pediatric alveolar rhabdomyosarcoma re-established the tumor-suppressive function of ILK.[30] These results suggest a conserved role for ILK as a tumor suppressor in certain mesenchymal lineage cells, and expression of PAX3-FKHR in these cells rescued the dampened growth induced by ILK overexpression, as in embryonal rhabdomyosarcoma cells.[30]

The ability to inhibit the ILK activity raised interest in ILK as a target for anticancer therapy. Three cellular inhibitors of ILK signaling, including phosphatase and tensin homolog deleted on chromosome 10 (PTEN), ILKAP, and β-Parvin (ParvB), are known. PTEN is a lipid phosphatase and a major antagonist of PI3K activity, and dephosphorylation of $PI(3,4,5)P_3$ by PTEN indirectly inhibits ILK activity.[31] ILKAP is a protein serine/threonine phosphatase of the PP2C family, and selectively binds to and inhibits ILK, blocking the phosphorylation of GSK3 S9.[32] ParvB, another key component of the PINCH–ILK–parvin complex, was noted to be a prognostic factor in some kinds of cancer.[33] In a study of breast cancer, ParvB inhibited ILK kinase activity and anchorage-independent cell growth, and the loss of ParvB expression can upregulate ILK activity in tumors.[34]

Some recent studies have also noted that ILK inhibitors, in combination with conventional chemotherapeutic agents such as gemcitabine or cisplatin, may be efficacious in cancer patients[35,36]; increased survival was observed in these patients, which was significantly more than the survival in those receiving either treatment alone. Most recently, studies on a xenograft model of EGFR-resistant human hepatoma-cell lines found that transfection with kinase-inactive ILK increased the sensitivity of cells to the EGFR inhibitors, which implied that the inhibition of ILK activity might overcome the resistance to EGFR inhibitors.[37]

ILK is involved in EMT

ILK is identified as an important mediator of EMT, and the increase of ILK activity is also suggested as a molecular marker for EMT.[4,14] EMT is a process through which cells lose epithelial properties, such as cell–cell adhesion and basoapical polarity, and gain mesenchymal properties, such as increased ability to migrate and invade through ECM proteins[38]; it describes a series of events involving the alterations in morphology, cellular architecture, adhesion, and migration capacity. It plays a role in three main processes, namely, embryogenesis, tissue repair, and cancer invasion/metastasis.[39]

Connecting with integrin, ILK downregulates E-cadherin expression[27] and is required for Transforming Growth Factor-β (TGF-β)-induced EMT.[40] In response to external triggers, ILK converges those signals into the Rho family GTPases,[41,42] and β1 integrins also activate RhoA and Rac1, which leads to the disruption of cadherin-mediated adhesions.[43] Together, these studies illustrate the role of ILK in cross-regulation between E-cadherin and integrins.[44]

Pathological overexpression of ILK results in down regulation of E-cadherin, and nuclear accumulation of β-catenin, and activation of other mesenchymal genes through nuclear factor (NF)-κB.[14,27,45] In a recent study of the overexpression of ILK on the human colorectal cancer cell line SW480, the cells stably overexpressing ILK underwent EMT, as indicated by mesenchymal morphology, decreased expression of E-cadherin, and increased expression of mesenchymal markers, as well as a dramatically promoted ability of migration and invasion *in vitro*.[46] Furthermore, applying the NF-κB inhibitor or NF-κB p65 small interfering RNA (siRNA) significantly restored the reduced E-cadherin level in these ILK-overexpressing cells, suggesting that ILK-mediated EMT is dependent on NF-κB activation.[46]

In a similar manner, inhibition of ILK signaling may reverse the EMT process and hinder cancer growth. Human ribonuclease inhibitor (RI), a cytoplasmic acidic protein that inhibits RNase A and angiogenin activities, was shown to inhibit growth and metastasis in some cancer cells.[47] In human clinical specimens, bladder cancer with a high metastasis capability shows higher expression of vimentin, Snail, Slug, and Twist, and lower expression of E-cadherin and RI. However, overexpression of RI would decrease the ILK expression, reduce phosphorylation of the ILK downstream signaling targets p-Akt and p-GSK3β, and reverse the process of EMT. Specifically, overexpression of RI induces upregulation of E-cadherin and inhibits the expression of MMP-2, MMP-9, and other mesenchymal markers, such as N-cadherin, Snail, Slug, vimentin, and Twist *in vitro*. Overexpression of RI also inhibits cell proliferation, migration, and invasion; alters cell morphology and adhesion; and leads to the rearrangement of the cytoskeleton *in vitro*.

Therefore, RI may inhibit the development of bladder cancer through inhibition of ILK signaling and reversal of the EMT process.[47]

In a study of tongue cancer, tumors with a high metastasis capability showed higher expression of ILK and mesenchymal markers, such as vimentin, Snail, Slug, and Twist, as well as lower expression of E-cadherin in clinical specimens. However, ILK siRNA inhibited EMT, cell proliferation, migration, and invasion, as well as changed cell morphology.[48] The study also demonstrated that ILK siRNA inhibited phosphorylation of downstream signaling targets Akt and GSK3β, as well as reduced the expression of MMP-2 and MMP-9, leading to the suppression of tumorigenesis and metastasis *in vivo*.[48]

ILK plays roles in vascular biology

New vessel formation is a dynamic process of interactions between endothelial cells (ECs) and endothelial progenitor cells (EPCs) and between each of them and the ECM.[49] As ILK plays a pivotal role in ECM-mediated signaling, studies revealed that ILK is a key molecule in the vascular biology, particularly the function, structure, and survival of ECs, and the process of neovascularization.[50]

In a study using the model of human umbilical vein ECs, endogenous ILK expression was decreased in various stress conditions, and retarded capillary tube formation was noted after a brief anchorage deprivation. However, adenoviral *ILK* gene transfer in ECs and EPCs reversed the decrease in cell survival signals, and significantly accelerated the functional recovery after reattachment. In the nude mice hindlimb ischemia model, the ILK-overexpressing EPCs significantly improved blood flow recovery and prevented limb loss.[51] As the ILK/Akt/GSK3β axis is activated in ECs and EPCs, it is speculated that ILK functions as an effecter of PI3K signaling, which regulates protein kinase B/Akt activity positively and GSK3 activity negatively.[52]

Possible role of ILK in mitosis

Recently a global analysis of the ILK "interactome" found that several centrosomal and mitotic-spindle proteins are binding partners of ILK, which implied that ILK might be important for mitotic-spindle assembly and have some roles in mitosis.[15,53] Further study demonstrated that ILK could be found in focal adhesions and centrosomes simultaneously in interphase cells, and ILK inhibition induced dramatic defects in mitotic-spindle organization, which indicates that ILK may be important for mitotic-spindle assembly.[54] Removal of ILK from hepatocytes *in vivo* has also been shown to cause mitotic defects.[55] It is likely that due to its role in mitosis, ILK can have some prospects of application in cell-based therapy against cancer.[15]

ILK's role in reproduction

ILK protein is highly detectable in placental tissue samples throughout gestation and highly expressed in human trophoblast during the 1st trimester of pregnancy. In a recent study, the dominant negative ILK trophoblast cell line showed dramatically reduced migration into wounds compared to cells expressing wild-type ILK, indicating that the ILK may promote trophoblastic cell migration during this period of development.[25]

In patients of pre-eclampsia, a specific hypertensive and vascular complication in pregnancy, both the expression of ILK and the number of EPCs (precursors of ECs) were diminished. While EPCs were transfected with the *ILK* gene, growth and angiogenic activities of EPCs were enhanced.[56] Another study showed that transfection with *ILK* also enhanced the proliferative, migratory, and angiogenic capabilities of EPCs, and promoted VEGF production.[57] These results imply that the *ILK* gene transfection is effective in augmenting the angiogenic activity of EPCs, hence a therapeutic potential for cell-based gene therapy in patients with pre-eclampsia.[57]

Conclusion and perspectives

ILK is now considered as a complex, multifunctional protein that dynamically regulates signals derived from integrin–matrix interactions or serine/threonine kinase activity through intermolecular interactions and kinase activity. ILK regulates cell–matrix interactions, cytoskeletal organization, and cell signaling with indispensable roles in embryonic development, and may also be important for mitotic-spindle assembly. Therefore, ILK has many important contributions to various cellular processes, including survival, proliferation, differentiation, adhesion, migration, and contractility. Inhibition of ILK causes proliferative defects, induces cell-cycle arrest and apoptosis, and is embryonically lethal. Increased ILK activity may promote EMT; induce a transformed, tumorigenic phenotype; and enhance VEGF expression.[5] In addition, as ILK plays some role in the activation of EPCs and neovascularization, strategies targeting ILK overexpression may be highly effective in cell-based therapeutic vasculogenesis in ischemic area.[51]

Greater expression of ILK was frequently noted in human malignancies, which has emerged as a valid therapeutic target in cancer. Specific inhibitors of ILK kinase activity have exhibited growth arrest and apoptosis in cells *in vitro* and *in vivo*, providing support for ILK as a therapeutic target in human cancer.[15] Of crucial importance, ILK inhibitors were tolerated well and exhibited no apparent toxicity in these studies.

It will be of great interest to delineate the exact functions and targets of ILK in centrosomes during the mitotic process, as mitotic defects are hallmarks of malignant cells, and due to its role in mitosis ILK can have some prospects in cell-based therapy against cancer.[15] However, it is still uncertain whether the centrosomal ILK has similar signaling targets (such as Akt, GSK3, and catenin) to conventional ILK known in focal adhesions, and the diversities of ILK function seem to be cell and/or tissue specific and dependent on contextual cues. Future breakthroughs can arise from an in-depth analysis of the many direct and indirect interactions associated with ILK in this field.

After all, the roles of ILK in reproductive sciences were underexplored. Although the function of ILK on trophoblastic cells and EPCs in pre-eclampsia have been studied in some publications, the possible roles of ILK in other interesting tissues such as the decidual cells and the ectopic endometrial cells of endometriosis and adenomyosis have merit for further investigation. The current standard of diagnosis and treatment of endometriosis is through the laparoscopy,[58,59] in which a tissue biopsy is usually available for further research. Many investigators believe that the eutopic endometrium and embedded immune-related cells may exhibit altered characteristics and interactions,[60] which may also affect the implantation process of the embryo. Besides, one potential cause of reproductive failures such as infertility and recurrent miscarriage may arise from the endometrial defect of cell communication or adhesion functions.[61] The exact mechanism of pathogenesis is still elusive, and the possible role of ILK in cell adhesion, EMT, or abnormal function of mitosis and proliferation would be interesting topics for further investigation.

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